acterized, as has release of corepression by all-*trans*-retinoic acid (ATRA) in therapy of APL. However, the effect of cleavage on these biological activities has not yet been determined. As a counterpoint, it should be noted that our current understanding of pathophysiology of PML-RARα-mediated leukemia has been predicated on expression of the full-length protein, and subsequent analyses may need to take into account activities of the cleaved products. Second, these experiments were performed using a bcr-1 PML-RARα breakpoint variant. APL may also be associated with a bcr-3 PML-RARα variant that lacks the V420 and V432 NE cleavage sites. The authors show that this variant is also cleaved by NE, but at different sites. Although this may indicate that cleavage is the important event rather than the site of cleavage, it complicates mutational analysis designed to test the hypothesis that the cleavage sites are critical for leukemogenesis. Third, although the available data argues against post-lysis artifacts, this is very difficult to definitively exclude short of mutating the putative cleavage sites, and as noted above this may be difficult given the presence of several sites. Fourth, it is not clear where or how NE gains access to PML-RARα, as they are thought to reside in different cellular compartments. Last, the human APL cell line NB4 containing the PML-RARα expresses the full-length PML-RARα fusion protein, and lacks NE activity. These data provide further correlation between NE activity and cleavage, but raise the question of why a leukemic cell line derived from a primary leukemia lacks this activity if it is critical for leukemogenesis.

Nonetheless, these findings are provocative, and warrant additional experimentation to validate the findings, and to determine the extent to which these observations can be extrapolated to other leukemogenic fusion genes. One enticing possibility is that proteolytic processing plays a more important role than previously appreciated in human leukemias. In addition to PML-RARα, there is a predilection among other leukemogenic fusion oncogenes for association with specific AML subtypes. For example, MLL fusions are frequently associated with myelomonocytic leukemias—it may be of interest to see if there are differences in MLL cleavage by Taspase 1 in this cellular context. Perhaps the most intriguing aspect of these two reports is the unexpectedly important role that proteolytic processing plays in these developmental and leukemogenic processes. Finally, these data suggest that certain proteases should be explored as potential therapeutic targets in leukemia.

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Selected Reading

Armstrong, S.A., Staunton, J.E., Silverman, L.B., Pieters, R., den Boer, M.L., Minden, M.D., Sallan, S.E., Lander, E.S., Golub, T.R., and Korsmeyer, S.J. (2002). Nat. Genet. *30*, 41–47.

Ayton, P.M., and Cleary, M.L. (2001). Oncogene *20*, 5695–5707.

Ayton, P.M., and Cleary, M.L. (2003). Genes Dev. *17*, 2298–2307.

He, L.S., Tribioloi, C., Rivi, R., Peruzzi, D., Pelicci, P.G., Soares, V., Cattoretti, G., and Pandolfi, P.P. (1997). Proc. Natl. Acad. Sci. USA *94*, 5302–5307.

Hsieh, J.J., Cheng, E.H.-Y., and Korsmeyer, S.J. (2003a). Cell, this issue, 293–303.

Hsieh, J.J., Ernst, P., Erdjumentt-Bromage, H., Tempst, P., and Korsmeyer, S.J. (2003b). Mol. Cell. Biol. *23*, 186–194.

Lane, A.A., and Ley, T.J. (2003). Cell, this issue, 305–318.

Minucci, S., Monestiroli, S., Givara, S., Ronzoni, S., Marchesi, F., Insigna, A., Diverio, D., Gasparini, P., Capillo, M., Colombo, E., et al. (2002). Blood *100*, 2989–2995.

Westervelt, P., and Ley, T.J. (1999). Blood *93*, 2143–2148.

Yeoh, E.J., Ross, M.E., Shurtleff, S.A., Williams, W.K., Patel, D., Mahfouz, R., Behm, R.G., Raimondi, S.C., Relling, M.V., Patel, A., et al. (2002). Cancer Cell *1*, 133–143.

Yu, B.D., Hanson, R.D., Hess, J.L., Horning, S.E., and Korsmeyer, S.J. (1998). Proc. Natl. Acad. Sci. USA *95*, 10632–10636.

MoMLV Reverse Transcriptase Regulates Its Own Expression

A precise ratio of Gag:Gag-Pol expression is required for assembly of infectious retroviral virions. In this issue of *Cell*, Orlova et al. show that MoMLV reverse transcriptase binds the translation release factor eRF1, and that this interaction promotes translation readthrough to make Gag-Pol.

The retroviral gene *gag* is expressed both as an independent polyprotein and as a fusion with the polyprotein encoded by the gene *pol*. The link between the structural components of the virion found in Gag and the viral enzymes found in Pol ensures that these enzymes are packaged in newly formed viral particles. But why does the virus require two forms of Gag? Exclusive expression of Gag results in the assembly and release of virus-like particles. However, these particles fail to infect cells because they lack the enzymes required for maturation of the virion and for replication. On the other hand, overexpression of Gag-Pol inhibits assembly, possibly due to the steric constraints of having Pol on the C terminus of Gag (Swanstrom and Wills, 1997), and can prevent the packaging of two copies of the viral RNA (Shehu-Xhilaga et al., 2001). Thus, a delicate balance between the expression of Gag and Gag-Pol is critical for infection and is carefully maintained by most retroviruses at a ratio close to 20:1(Shehu-Xhilaga et al., 2001).

Because of the importance of the Gag:Gag-Pol ratio for the assembly of infectious virions, retroviruses have developed several strategies to switch between Gag and Gag-Pol expression (Gale et al., 2000). For human immunodeficiency virus (HIV), the *gag* and *pol* genes share an overlapping reading frame, and a −1 frameshift near the end of *gag* permits readthrough to the *pol* gene. For Moloney murine leukemia virus (MoMLV), the *gag* and *pol* genes are in-frame, and suppression of the stop codon separating them permits the expression of Gag-Pol. Although much has been learned about the viral

mRNA requirements for *gag-pol* readthrough, less is known about the regulatory mechanisms that control this process.

In this issue of *Cell*, Orlova et al. (2003) identify eukaryotic release factor 1 (eRF1) as a cellular protein that interacts with MoMLV reverse transcriptase (RT), a viral enzyme encoded by *pol*. eRF1 has an integral role in the normal termination of translation (Kisselev et al., 2003). When the moving ribosome encounters a stop codon, eRF1 binds in the A-site and, with the ribosomal RNA, facilitates the cleavage of the peptidyl-tRNA bond by an unknown mechanism to release the newly synthesized protein. Given the role of eRF1 in translation termination, Orlova et al. set out to further investigate the potential interaction between eRF1 and RT and whether or not RT is involved in the regulation of its own expression.

The authors first present evidence supporting an interaction between RT and eRF1 both in vitro and in vivo. In vitro, RT is retrieved from solution by an eRF1-glutathione S transferase (GST) fusion bound to glutathione beads. Both domains of RT, the DNA polymerase domain and the RNase H domain, appear to interact with eRF1 by this assay, although the interaction with the DNA polymerase domain appears to be weaker than the interaction with full-length RT. In vivo, overexpression of eRF1 leads to its incorporation into viral particles, suggesting that the translation release factor is being recruited by viral proteins. When portions of the RT gene, or the entire RT gene, are deleted, eRF1 is not incorporated into the viral particles, suggesting that eRF1 interacts specifically with RT.

After confirming the putative interaction between RT and eRF1, Orlova et al. examined the effect of RT on Gag-Pol readthrough with exciting results. Using a reporter construct consisting of the *gag-pol* junction flanked by two different luciferase genes, they observed that an increase in RT expression in vivo leads to an increase in expression of the luciferase fusion protein. The effect of RT on readthrough at the *gag-pol* junction is countered by the simultaneous overexpression of eRF1, further supporting the interaction between these proteins. To investigate the role of RT on the expression of Gag-Pol in MoMLV infected cells, the authors transfected cells with proviral DNA lacking the RT gene. In the absence of RT, the expression level of Gag-Pol is not detectable by Western blot analysis, whereas the expression of Gag is equal to that observed in cells transfected with the wild-type provirus. These combined observations, that increasing RT can increase readthrough at the *gag-pol* junction and that the removal of RT prevents readthrough, strongly support a role for RT in the regulation of its own synthesis.

Is the regulatory role of RT related to its interaction with eRF1? To answer this question, Orlova et al. isolated mutants of RT that fail to interact with eRF1. Similar to the effect of removing the RT gene entirely, cells transfected with proviral DNA containing these RT mutations failed to express the Gag-Pol protein. The Gag protein was expressed at wild-type levels in these cells but remained unprocessed, consistent with the fact that protease is encoded by *pol*. The viral particles produced from these mutant proviral vectors, and from the RT-minus proviral vector, were harvested and used to infect new cells. Whereas virions derived from the RT-minus and eRF1 interaction-deficient mutants failed to produce any progeny virus even after two weeks, viral particles derived from the wild-type vector were highly infectious. Thus, the effect of RT on *gag-pol* readthrough is dependent on an interaction between RT and eRF1.

By demonstrating that an MoMLV-RT upregulates its own expression through a specific interaction with the host translation termination machinery, Orlova et al. have discovered a new way in which retroviruses manipulate cellular processes to their advantage. This exciting discovery raises many questions about the mechanism by which the RT-eRF1 interaction affects stop codon suppression; like all interactions in such feedback loops, the key answers lie in the binding equilibriums. What is the effect of RT on the affinity of eRF1 for mRNA, the ribosome, and the other translation release factor, eRF3? What is the affinity of RT for eRF1, and do other cellular and viral cofactors play a role in this regulatory circuit? A biophysical examination of this system could explain even further how MoMLV masters the delicate regulation of Gag and Gag-Pol expression.

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Selected Reading

Gale, M., Jr., Tan, S.L., and Katze, M.G. (2000). Microbiol. Mol. Biol. Rev. *64*, 239–280.

Kisselev, L., Ehrenberg, M., and Frolova, L. (2003). EMBO J. *22*, 175–182.

Orlova, M., Yueh, A., Leung, J., and Goff, S.P. (2003). Cell *115*, this issue, 319–331.

Shehu-Xhilaga, M., Crowe, S.M., and Mak, J. (2001). J. Virol. *75*, 1834–1841.

Swanstrom, R., and Wills, J.R. (1997). Synthesis, assembly and processing of viral proteins. In Retroviruses, J.M. Coffins, S.H. Hughes, and H.E. Varmus, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 263–334.

HtrA2/Omi, a Sheep in Wolf's Clothing

Mammalian mitochondrial HtrA2/Omi was originally described as an apoptosis inducer, but rather than having extra cells, mice with mutant HtrA2/Omi suffer from a neurodegenerative disease due to progressive mitochondrial damage. This suggests that instead of promoting cell death by antagonizing inhibitor of apoptosis (IAP) proteins, the primary function of HtrA2/Omi is to handle misfolded proteins in the mitochondria.